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# Prostaglandin $E_2$ Alleviates Cyclosporin A-Induced Bone Loss in the Rat

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## ABSTRACT

Cyclosporine A (CsA) administered to the male and female rat produces high-turnover osteopenia. Prostaglandins have both bone-resorbing and bone-forming properties, but administration of prostaglandin $E_2$ ($PGE_2$) to the rat in vivo produces a net increase in cancellous bone. To investigate the effects of $PGE_2$ on CsA-induced alteration in bone mass, 43 male Sprague-Dawley rats (9 weeks old) were administered 15 mg/kg of CsA by oral gavage and/or 6 mg/kg of $PGE_2$ by subcutaneous injection daily for 21 days according to the following protocol: group A was an age-matched control; group B received CsA only; group C received $PGE_2$ only; and group D received CsA and $PGE_2$. Serum was assayed on days 0, 7, 14, and 21 for bone gla protein (BGP), PTH, and 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D]. A computerized image analysis system was used for bone histomorphometry of the proximal tibial metaphysis after double tetracycline labeling. Compared to control animals (group A), treatment with CsA alone (group B) and $PGE_2$ alone (group C) significantly elevated BGP levels. Combination therapy (group D) resulted in BGP levels that were significantly higher on days 7 and 14 than with either agent alone. 1,25-$(OH)_2$D was significantly elevated in the CsA group only (group B). Therapy with CsA alone (group B) resulted in a significant osteopenia. The concurrent administration of $PGE_2$ with CsA (group D) alleviated the altered bone mass induced by CsA alone by adding a significant amount of additional bone. This report confirms and extends the current knowledge of the different effects of CsA and $PGE_2$ on bone mineral metabolism and demonstrates that $PGE_2$ can alleviate the deleterious effects of CsA on bone.

## INTRODUCTION

CYCLOSPORIN A (CsA), an immunosuppressive agent that is frequently used in the management of organ transplantation and autoimmune states, alters the immune response by inhibiting cytokine production and release and T lymphocyte activation.[1-3] It has been recognized that cytokine production by cells of the immune system affect bone metabolism at a local level and may therefore be important in the regulation of bone remodeling.[4] Thus, it is not surprising that CsA affects bone mineral metabolism, inducing a state of high-turnover bone loss in rats, dependent on the dose and duration of treatment.[5-7] This osteopenic state occurs in the absence of significant renal failure, and is characterized by elevated serum bone gla protein (BGP) levels.

Prostaglandins (PGs) are important local regulators of cellular function and have been found to have complex and biphasic effects on both bone formation and resorption in vivo and in vitro.[8] In the presence of physiologic concentrations of cortisol, the major effect of prostaglandin $E_2$ ($PGE_2$) in vitro is to stimulate bone formation.[9] Clinically, systemic prostaglandin $E_1$ ($PGE_1$) is reported to stimulate new bone formation at periosteal surfaces in neo-

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nates,[10-14] and $PGE_2$ and related compounds stimulate the formation of new woven bone trabeculae in long bones of both young and aged rats.[15-18] Similarly, in dogs, $PGE_2$ is a potent stimulator of cancellous and cortical bone formation.[19,20] Although PGs have immunosuppressive properties,[21,22] CsA actually inhibits PG production in the kidney,[23] probably by inhibition of the release of substrate arachidonic acid from cell membranes.

Because PGs stimulate bone formation in vivo, we hypothesized that the concurrent administration of $PGE_2$ with CsA might be beneficial to bone. The results of such an experiment may be helpful in a number of ways. First, the pathophysiology of CsA-induced bone disease may be better understood with regard to the influence of PGs. Second, it may show that patients receiving both CsA and PGs for the alleviation of renal disease, as was recently reported,[24] may have a reduced risk of bone disease. Finally, a new therapeutic option for treating osteopenic states may be introduced. Therefore, an in vivo experiment examining the effects of CsA and $PGE_2$ on bone mineral metabolism in the rat was conducted.

## MATERIALS AND METHODS

### *Animals*

A group of 43, 9-week-old male Sprague-Dawley rats weighing approximiately 300 g were obtained from Charles River Laboratories (Wilmington, MA). All rats were housed under similar conditions and maintained on a diet of Agway Prolab RMH 3000 (0.75% calcium, 0.85% phosphorus, 1045 IU/kg of vitamin $D_3$, 22.5% protein, 5.5% fat, and 52% carbohydrate) and tap water ad libitum.

### *Drugs*

CsA solution (Sandoz, Inc., East Hanover, NJ) at a concentration of 100 mg/ml in 12.5% alcohol by volume and olive oil was diluted in alcohol-olive oil vehicle to obtain a final concentration of 15 mg/ml. $PGE_2$ (Upjohn Pharmaceuticals, Kalamazoo, MI) was dissolved in alcohol to obtain a stock solution of 10 mg/ml. This stock was diluted with 0.1 M phosphate buffer (2.20 g $NaH_2PO_4 \cdot H_2O$ + 1.69 g $Na_2HPO_4 \cdot 7H_2O$ per liter) to obtain fresh aliquots of 6 mg/ml each day. An equivalent volume of alcohol was dissolved in buffer to obtain $PGE_2$ vehicle. The dose of $PGE_2$ used in this experiment was equivalent to a dose used to obtain maximal trabecular bone formation in previous experiments.[15,18]

### *Experimental protocol*

Rats were randomly divided into four groups and administered vehicles, CsA, and/or $PGE_2$ for 21 days according to the following protocol: group A (control) were age-matched controls and received CsA vehicle (100 μl per 100 g body weight) by gavage plus $PGE_2$ vehicle (100 μl per 100 g body weight) by subcutaneous (sc) injection into the back of the neck ($n$ = 11); group B (CsA) received CsA (15 mg/kg) by gavage and $PGE_2$ vehicle ($n$ = 11); group C ($PGE_2$) received $PGE_2$ (6 mg/kg) and CsA vehicle ($n$ = 10); and group D (CsA and $PGE_2$) received CsA (15 mg/kg) and $PGE_2$ (6 mg/kg) by sc injection ($n$ = 11).

All rats received tetracycline hydrochloride (Achromycin; Lederle Laboratories, Pearl River, NY) 15 mg/kg body weight by intraperitoneal injection on days 9 and 19 for histomorphometric determinations of dynamic parameters of bone remodeling.

Experimental procedures were reviewed and approved by an institutional animal care committee in accordance with current National Institute of Health policy.

### *Blood collection and analysis*

Rats were weighed and bled on days 0, 7, 14, and 21 after 10 mg per 100 g body weight ketamine hydrochloride (Ketaset; Aveco Co., Inc., Fort Dodge, IA) and 0.1 mg per 100 g body weight acepromazine maleate (Techamerica Group Inc., Elwood, KS) intramuscular administration. All blood samples were obtained via orbital sinus venous puncture except when rats were sacrificed by cardiac puncture on day 21. Blood samples were centrifuged and the sera stored at −70°C.

Serum bone gla protein was measured by radioimmunoassay (RIA) using a modification of a previously described assay.[25] Antibody to rat BGP (kindly donated by Dr. K. Nishimoto, Department of Biochemistry, University of Kentucky, KY) was used in a final dilution of 1:25,000, and purified rat BGP was used for the preparation of standards and $^{125}$I-labeled tracer. After a 24 h equilibrium assay, antibody-BGP complexes were separated from free $^{125}$I-labeled BGP with goat antirabbit gamma globulin (Calbiochem, LaJolla, CA). The lower limit of detection for the assay is 0.5 ng/ml, and the intra- and interassay coefficients of variation are 5.9 and 8.9%, respectively.

Serum immunoreactive parathyroid hormone (PTH) was measured by RIA using a commercially available kit (N-terminal PTH; Nichols Institute, San Juan Capistrano, CA) with an antiserum to human PTH-(1-34) that cross-reacts with rat PTH-(1-34). The validity of this assay was confirmed in our laboratory by showing low values for serum immunoreactive PTH in parathyroidectomized rats and elevated values in EDTA-induced hypocalcemic rats. The characteristics of this assay have been published.[26] Intra- and interassay coefficients of variation are 8.2 and 10.4%, respectively.

1,25-Dihydroxyvitamin D [1,25-$(OH)_2$D] was assayed using a commercially available radioreceptor assay kit (Incstar, Stillwater, MN), specific for both 1,25-$(OH)_2D_2$ and 1,25-$(OH)_2D_3$. Intra- and interassay coefficients of variation are 3.5 and 8.5%, respectively.

### *Histologic techniques*

Following sacrifice, the right tibia was removed from each rat, dissected free of soft tissue, and fixed in 70% ethanol. The proximal third of each tibia was stained in Villanueva bone stain (Polysciences, Inc., Warrington, PA) for 5 days. Specimens were dehydrated by sequential changes

in ascending concentrations of ethanol and acetone and then embedded in methyl methacrylate (Eastman Organic Chemicals, Rochester, NY). Frontal sections (230 μm thick) of the proximal tibia were cut through the tibial eminence with a precision bone saw. Two sections from each tibia were ground to 100 μm thick and microradiographed on Kodak spectroscopic plates (649-0; Eastman Kodak, Rochester, NY).[27] The 100 μm thick sections were mounted on plastic microscope slides with cyanoacrylate adhesive (910 adhesive; Commercial Plastics, Salt Lake City, UT) and further ground to a thickness of 20 μm for static and dynamic histomorphometric measurements.

Microradiographs and a quantitative television microscope (QTM; Cambridge Instruments, Cambridge, England) coupled to a DEC 11/03 microcomputer were employed to measure metaphyseal tissue and hard tissue parameters. Measurements were performed on zone 1 (1 mm distal to the growth plate-metaphyseal junction) and zone 2 between 1 and 4 mm distal to the growth plate-metaphyseal junction.[28] The percentage trabecular area and trabecular width, number, and separation were calculated according to Parfitt et al.[29,30] and Jee et al.[31] (Table 1).

Sections 20 μm and a digitizing image analysis system were used to determine selected static and dynamic metaphyseal cancellous bone histomorphometric indices. The system consists of an epifluorescent microscope, a digitizing pad (Summagraphic, Fairfield, CT) coupled to an Apple MacIntosh SE computer, and a morphology program named Stereology (KSS Computer Systems, Magna, UT). The metaphyseal area, trabecular area and perimeter, eroded perimeter, single- and double-labeled perimeter, and interlabeling distance of double-fluorescent labels were measured in zone 2 at ×156 or ×312 magnification.[28,31] Percentage trabecular area, trabecular number and width, percentage eroded and labeling perimeter, ratio of eroded to labeled perimeter, adjusted mineral apposition rate, and bone formation rates were calculated.[29-33] The measured and derived parameters are listed and defined in Table 1.

### *Statistical methods*

Statistics were calculated using the statistics packages SPSS/PC* (SPSS, Inc., Chicago, IL) and SAS (SAS Institute, Inc., Cary, NC). Repeated-measures analysis for rat weight and biochemical data was performed with multivariate analysis of variance (MANOVA). Analysis of the bone histomorphometry was performed with a 2 × 2 factorial analysis of variance (ANOVA), including a priori pairwise contrasts. Both models included interactions between CsA and $PGE_2$. The test of the CsA × $PGE_2$ interaction assesses whether the magnitude of any alterations in the biochemical or bone histomorphometric parameters induced by CsA depends on the presence or absence of $PGE_2$ The pairwise contrasts test differences in mean bone parameters between specific groups of rats in the context of the 2 × 2 factorial experiment. A $p$ value $< 0.05$ was considered a significant difference. All values are reported as the mean ± standard error of the mean (SEM) in both tables and figures. Even when the 2 × 2 interaction term for the interaction of CsA and $PGE_2$ is not significant, the differences between groups are reflected accurately by the pairwise contrasts.

## RESULTS

### *Body weight*

Rats receiving CsA [groups B (CsA) and D (CsA and $PGE_2$)] lost a significant amount of weight during the first week compared to rats not receiving CsA [groups A (control) and C ($PGE_2$); $p < 0.05$]. Thereafter, all groups gained weight at comparable rates, although body weight in the groups receiving CsA [groups B (CsA) and D (CsA and $PGE_2$)] was significantly lower than in control animals (group A) at all time points. Over time, there were no significant differences between groups A (control) and C ($PGE_2$) or B (CsA) and D (CsA and $PGE_2$). In the factorial model, the effect of CsA on weight over time did not depend on the presence or absence of $PGE_2$ (Fig. 1).

### *Biochemical analysis*

Serum BGP levels were significantly elevated in all treatment groups compared to group A (control) rats from day 7 onward (Fig. 2). Additionally, BGP levels in the combination group D (CsA and $PGE_2$) was significantly elevated compared to the group receiving CsA only (group B) on day 7 and to the groups receiving either CsA or $PGE_2$ (groups B and C) on day 14. In the repeated-measures model there was a significant three-way interaction among time, CsA, and $PGE_2$, indicating that the effect of CsA on BGP over time was dependent on the presence or absence of $PGE_2$ ($p < 0.002$).

Serum 1,25-$(OH)_2$D was significantly elevated in the CsA-treated rats only (group B) compared to all the other groups (Fig. 3). There was a significant three-way interaction among time, CsA, and $PGE_2$, indicating that the effect of CsA on serum 1,25-$(OH)_2$D over time was dependent on the presence or absence of $PGE_2$ ($p = 0.0008$).

There were no significant alterations in PTH throughout the 21 day experiment, except for a reduced level in group C ($PGE_2$) on day 14 compared to all the other groups only (Fig. 4). However, the effect of $PGE_2$ on PTH over time did not depend on the presence or absence of CsA.

### *Proximal tibial metaphyseal (PTM) cancellous bone histomorphometry*

*Microradiographic Appearance of Proximal Tibial Metaphyses:* CsA-treated bone had an obvious reduction in trabecular bone throughout the PTM compared with age-matched controls (Fig. 5, B versus A). $PGE_2$ alone dramatically increased trabecular bone mass in the PTM (Fig. 5, C versus A). The combination of $PGE_2$ with CsA alleviated the bone loss induced by CsA (Fig. 5, D versus B) and added extra bone to the PTM (Fig. 5, D versus A).

*Analysis of Microradiographs (Zone 1):* Table 2 summarizes the effects of CsA and/or $PGE_2$ on trabecular

TABLE 1. HISTOMORPHOMETRIC MEASUREMENTS AND CALCULATIONS

| *Parameters* | *Code* | *Unit* | *Description/formula* |
|---|---|---|---|
| Static measurements and calculations (zones 1 and 2) | | | |
| Total tissue area | T.Ar | $mm^2$ | Zones 1 and 2[a] |
| Trabecular area | Tb.Ar | $mm^2$ | Total cancellous bone area within total area |
| Trabecular perimeter | Tb.Pm | mm | The perimeter of Tb.Ar |
| Percentage trabecular area | %Tb.A | % | Tb.Ar/T.Ar × 100 |
| Trabecular width | Tb.Wi | μm | (2000/1.199) × Tb.Ar/Tb.Pm |
| Trabecular number | Tb.N | No./mm | 1.199/2 × Tb.Pm/T.Ar |
| Trabecular separation | Tb.Sp | μm | (2000 × 1.199) × (T.Ar − Tb.Ar)/Tb.Pm |
| Dynamic measurements and calculations (zone 2) | | | |
| Single-labeled perimeter | Sl.Pm | mm | Length of trabecular surface with a single tetracycline label |
| Double-labeled perimeter | dL.Pm | mm | Length of trabecular surface labeled with 2 tetracycline labels |
| Interlabel width | Ir.L.Wi | μm | Distance between two tetracycline labels |
| Interlabel width (growth) | Ir.L.Wi.-G | μm | Distance between tetracycline labels in growth plate-metaphyseal junction region |
| Osteoid perimeter | O.Pm | mm | Length of trabecular surface covered with osteoid |
| Eroded perimeter | E.Pm | mm | Length of trabecular surface with Howship's lacuna |
| Labeled perimeter | L.Pm | mm | dL.Pm + sL.Pm/2 |
| Percentage labeled perimeter | %L.Pm | % | (dL.Pm + sL.Pm/2)/Tb.Pm × 100 |
| Percentage eroded perimeter | %E.Pm | % | E.Pm/Tb.Pm × 100 |
| Percentage osteoid perimeter | %O.Pm | % | O.Pm/Tb.Pm × 100 |
| Adjusted mineral apposition rate | Adj.MAR | μm/day | Ir.L.Wi/Interval × L.Pm/O.Pm |
| Bone formation rate 1 (per bone area) | BFR/BV | %/year | (dL.Pm + sL.Pm/2) × Adj.MAR/Tb.Ar × 365 × 100 |
| Bone formation rate 2 (per bone surface) | BFR/BS | μm/day × 100 | (dL.Pm + sL.Pm/2) × Adj.MAR/Tb.Pm × 100 |
| Bone formation rate 3 (per tissue area) | BFR/TV | %/year | (dL.Pm + *sL.Pm/2)* × Adj.MAR/T.Ar × 365 × 100 |
| Longitudinal growth rate | LGR | μm/day | Ir.L.Wi-G/interval |
| Eroded/labeled perimeter | E/L | Ratio | E.Pm/L.Pm |

[a]Zone 1, total tissue area up to 1 mm distal to the growth plate-metaphyseal junction; zone 2, total tissue area between 1 and 4 mm distal to the growth plate-metaphyseal junction.

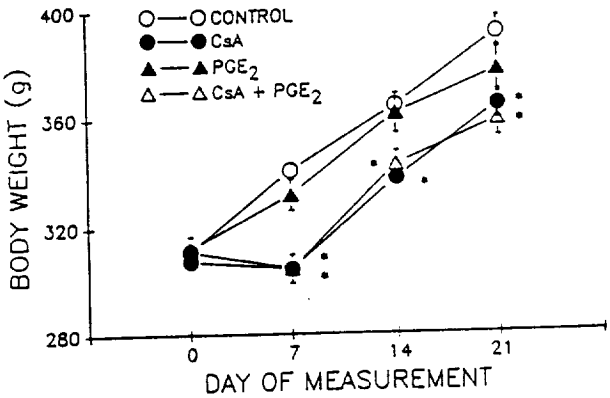


FIG. 1. Effect of CsA and/or $PGE_2$ on body weight in normal rats. Rats were weighed weekly. $^*p < 0.05$ versus control.

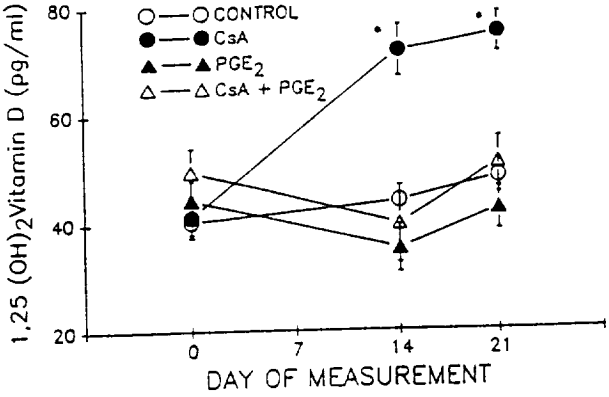


FIG. 3. Effect of CsA and/or $PGE_2$ on serum 1,25-$(OH)_2D$ in normal rats. 1,25-$(OH)_2D$ was measured on days 0, 14, and 21 using a radioreceptor assay specific for both 1,25-$(OH)_2D_2$ and 1,25-$(OH)_2D_3$ as detailed in Materials and Methods. $^*p < 0.05$ versus control.

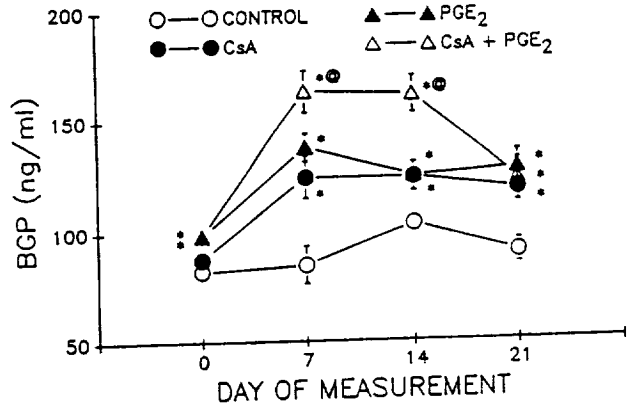


FIG. 2. Effect of CsA and/or $PGE_2$ on serum BGP in normal rats. BGP was measured using an in-house radioimmunoassay as detailed in Materials and Methods. $^*p < 0.05$ versus control; $@p < 0.05$ versus CsA group B. Differences on day 0 were accounted for in the factorial model.

bone area and structure in zone 1 of PTM. Zone 1 is the region 1 mm distal to the growth plate-metaphyseal junction that was designated primary spongiosa by Kimmel and Jee.[28] Rats treated with CsA alone (group B) had a significantly lower percentage of trabecular bone area, trabecular number, and primary spongiosa width and significantly higher trabecular separation than controls (group A). $PGE_2$ alone (group C) had exactly the opposite effect, producing significantly elevated percentage trabecular bone area, trabecular number, and primary spongiosa width and significantly reduced trabecular separation compared to the age-matched controls (group A). The concurrent administration of $PGE_2$ and CsA (group D) alleviated the bone changes induced by CsA alone (group B); no significant differences were found in percentage trabecular bone area, trabecular number, trabecular separation and trabecular width between the combination group (group D) and the age-matched controls (group A) by a pairwise comparison. The growth plate width was significantly depressed in both groups receiving $PGE_2$ compared to control.

*Analysis of Microradiographs (Zone 2):* Zone 2 is the region of secondary spongiosa (1–4 mm distal from the growth plate-metaphyseal junction) in the PTM. Similar results for zone 2 were demonstrated in 100 μm microradiographs (data not shown) and the 20 μm sections (see later).

*Static and Dynamic Parameters of 20 μm Sections (Zone 2):* Table 3 summarizes the results of the static histomorphometry in 20 μm sections from zone II. The percentage trabecular bone area, perimeter, and number were significantly reduced in CsA-treated animals (group B) compared to controls (group A), but trabecular separation was significantly increased compared to control animals. Again, $PGE_2$ administration alone (group C) had an effect opposite to that of CsA alone (group B), producing significant elevations in trabecular bone area, perimeter, width, and number and a nonsignificant reduction in trabecular separation compared to controls (group A). The concurrent administration of $PGE_2$ with CsA (group D) produced significantly increased trabecular bone area, perimeter, and number compared to both controls (group A) and the rats receiving only CsA (group B). Additionally, although the $PGE_2$ only (group C) and the combination group (group D) were not directly compared in the pairwise comparison, it can easily be appreciated that the combination group had less bone (percentage trabecular area) than the $PGE_2$-only group. Longitudinal growth rate was not significantly different among the four groups.

Table 4 summarizes the alterations that occurred in the dynamic histomorphometry of the secondary spongiosa. The CsA-treated group B had significantly elevated indices of bone formation and resorption compared to age-matched controls (group A); percentage labeling perimeter, percentage osteoid perimeter, percentage eroded pe-

rimeter, and the bone formation rates referent to trabecular bone area (BFR/BV) and bone surface (BFR/BS) were all significantly elevated. The ratio of eroded to labeling perimeter, however, was not significantly different between the CsA-treated rats (group B) and controls (group A).

The $PGE_2$-treated group C had significantly elevated labeling perimeters as well as the adjusted mineral apposition rates and all the bone formation rates compared to control (group A). These changes indicate a definite increase in bone formation. Resorption parameters (eroded perimeter and percentage eroded perimeter) were also significantly increased in this group compared to control (group A), indicating that $PGE_2$ administration also increased bone resorption. The ratio of eroded to labeling perimeter was not significantly different between the $PGE_2$-treated rats and the controls.

The combination group D (CsA and $PGE_2$) had significant elevations in absolute and percentage labeling, osteoid, and eroded perimeters compared to the age-matched controls (group A) in a similar fashion to the $PGE_2$-alone group. Most of these parameters were also significantly higher than in the CsA-treated group B. Additionally, the combination group D had a significant elevation in the ratio of eroded to labeled perimeter compared to the controls.

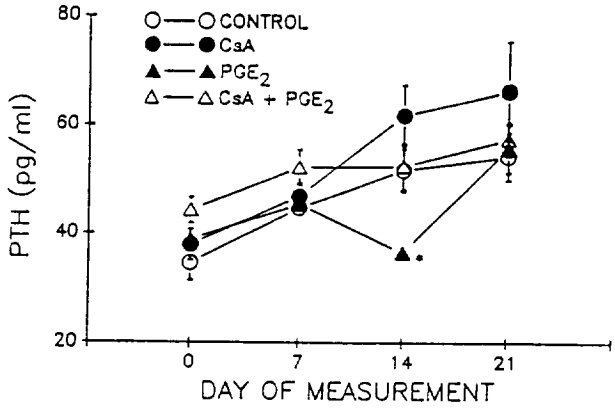


**FIG. 4.** Effect of CsA and/or $PGE_2$ on serum PTH levels in normal rats. Serum PTH was measured using a commercially available N-terminal PTH immunoassay as detailed in Materials and Methods. $*p < 0.05$ versus control.

## DISCUSSION

This study examines the effects of $PGE_2$ on CsA-induced alterations in circulating markers of bone mineral metabolism and proximal tibial trabecular bone histomorphometry. Rats that received CsA alone lost a significant amount of bone; rats that received $PGE_2$ alone had a significant increase in bone mass compared to age-matched controls. The concurrent administration of $PGE_2$ and CsA alleviated the bone loss induced by CsA by adding extra bone to the PTM.

The present report is the most detailed examination of the effects of CsA on trabecular bone. This laboratory reported previously that CsA produces a state of high-turnover bone loss in rats in vivo, with accelerated bone resorption exceeding bone formation.[5-7] Conversely, other investigators have reported increased bone formation and reduced bone resorption in rats receiving CsA.[34,35] Both these groups examined rat tail vertebrae, which has a higher percentage of cortical bone. Both microradiographic and microscopic histomorphometry in the present paper confirmed the reduced percentage trabecular bone

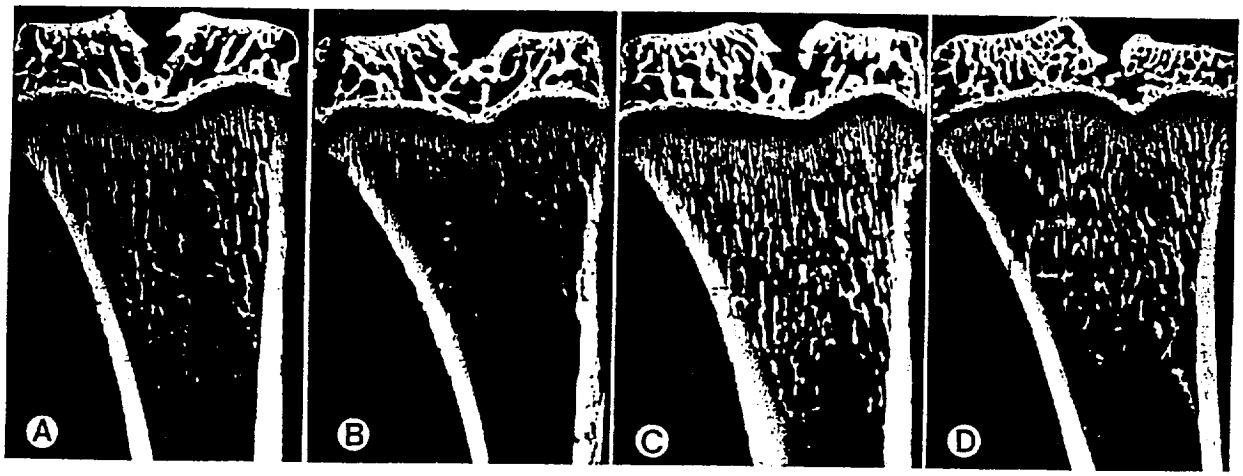


**FIG. 5.** Microradiographs showing the cancellous bone changes in proximal tibial metaphyses from age-matched controls (A), rats treated with 15 mg/kg/day of CsA for 21 days (B), rats treated with 6 mg/kg/day of $PGE_2$ for 21 days (C), and rats treated with a combination of CsA and $PGE_2$ in these doses for 21 days (D). A loss of cancellous bone occurred throughout the metaphysis in the CsA-treated bone (B). $PGE_2$ alone (C) increased trabecular bone mass in the proximal tibial metaphysis compared to control (A). The concurrent administration of $PGE_2$ with CsA prevented any CsA-induced bone loss and also added extra cancellous bone to the proximal tibial metaphysis (D). Original magnification = ×3.5.

area (equivalent to percentage trabecular bone volume) after CsA administration found in the previous experiments.

The final consequence of any factor affecting trabecular bone is an alteration in percentage trabecular bone area, which is a composite indication of trabecular width, trabecular number, and trabecular separation.[29] CsA treatment reduced the cancellous bone mass and architecture (Tables 2 and 4) by increasing the tissue-based percentage eroded perimeter by a factor of 2 (7.1 ± 1.0 versus 3.6 ± 0.4 % in controls), but its tissue-based bone formation rate was unchanged compared to controls (Table 4). This imbalance in resorption over formation reduced the trabecular number and increased the trabecular separation markedly but left the trabecular width unaltered (Table 3). These architectual changes suggest that CsA induced a massive resorption that wiped out the entire trabeculae, reducing trabecular number and surface; thus, the eroded perimeter value as reported is an underestimation of resorption because of the massive reduction in bone mass.

The action of the $PGE_2$ treatment confirms what was reported previously. $PGE_2$ activated bone remodeling and modeling in the formation mode; that is, formation exceeding resorption during remodeling and formation drifts to increase bone mass (Table 3).[15-20,36] The combination of CsA and $PGE_2$ was found to be 30% less effective than $PGE_2$ in maintaining and adding trabecular bone mass (Table 3) and 42% less effective in tissue-based bone formation rate (Table 4; CsA + $PGE_2$, 20.4 ± 1.8 versus 35.3 ± 4.6%/year), but an increased percentage in eroded perimeter (10.6 ± 0.6%) above that for $PGE_2$ (6.7 ± 0.6%) and CsA (7.1 ± 1.0%) treatment alone (Table 4). The increase in eroded surface could have been detrimental by reducing bone mass (reversible bone loss), but the anabolic effect of $PGE_2$-induced a net positive bone balance. Thus, the net result is a CsA + $PGE_2$ increase in trabecular bone mass by a factor of 4 over CsA alone and 72% over control rats (Table 3). In addition, trabecular architecture was only slightly inferior to that of $PGE_2$ alone in intact rats but much improved over CsA and control rats (Table 3). Therefore, the beneficial effects of PGE in alleviating CsA-induced bone loss was from its ability to increase bone mass.

The tissue-based bone formation rate was a better esti-

TABLE 2. MICRORADIOGRAPHIC HISTOMORPHOMETRY OF PROXIMAL TIBIAL METAPHYSEAL CANCELLOUS BONE, ZONE 1[a]

| *Tissue-based parameters* | *Control (A) (n = 11)* | *CsA (B) (n = 7)* | *$PGE_2$ (C) (n = 10)* | *CsA and $PGE_2$ (D) (n = 10)* | *Interaction term* |
|---|---|---|---|---|---|
| Trabecular area, % | 27.5 ± 2.0 | 18.6 ± 2.1[b] | 40.0 ± 2.3[d] | 32.1 ± 2.3[e] | 0.84 |
| Trabecular width, μm | 57.4 ± 2.8 | 52.5 ± 2.2 | 60.6 ± 2.9 | 59.6 ± 3.6 | 0.54 |
| Trabecular number per mm | 4.8 ± 0.3 | 3.6 ± 0.4[c] | 6.6 ± 0.2[d] | 5.4 ± 0.2[e] | 0.93 |
| Trabecular separation, μm | 225.3 ± 16.7 | 352.7 ± 48.2[d] | 132.4 ± 7.4[c] | 184.6 ± 10.9[e] | 0.07 |
| Primary spongiosa width, μm | 759.0 ± 19.8 | 553.5 ± 14.0[d] | 895.1 ± 24.0[d] | 739.2 ± 18.9[e] | 0.26 |
| Growth plate width, μm | 189.7 ± 10.0 | 174.3 ± 5.3 | 170.2 ± 4.5[b] | 161.0 ± 2.4[b] | 0.67 |

[a]Microradiographic histomorphometry of proximal tibial metaphyseal cancellous bone (zone 1) after rats were sacrificed on day 21. Group A = age-matched control, group B = cyclosporin A (CsA; 15 mg/kg/day by gavage), group C = prostaglandin $E_2$ ($PGE_2$; 6 mg/kg/day by subcutaneous injection), group D = CsA and $PGE_2$. All values = mean ± SEM.
[b]$p < 0.05$ versus control.
[c]$p < 0.01$ versus control.
[d]$p < 0.001$ versus control.
[e]$p < 0.001$ versus CsA group.

TABLE 3. STATIC HISTOMORPHOMETRY (ZONE 2) AND LONGITUDINAL GROWTH RATE[a]

| | *Control (A) (n = 11)* | *CsA (B) (n = 10)* | *$PGE_2$ (C) (n = 10)* | *CsA and $PGE_2$ (D) (n = 7)* | *Interaction term* |
|---|---|---|---|---|---|
| Trabecular area, % | 9.2 ± 1.3 | 4.1 ± 1.1[b] | 22.9 ± 1.9[d] | 15.8 ± 1.3[c,e] | 0.50 |
| Trabecular perimeter, mm | 38.5 ± 5.4 | 15.6 ± 4.3[c] | 63.8 ± 5.0[c] | 58.8 ± 5.2[c,e] | 0.09 |
| Trabecular width, μm | 43.7 ± 1.9 | 48.5 ± 2.4 | 59.4 ± 2.6[d] | 52.9 ± 1.3[c] | 0.01 |
| Trabecular number, mm | 2.1 ± 0.2 | 0.8 ± 0.2[d] | 3.8 ± 0.2[d] | 3.0 ± 0.2[c,e] | 0.42 |
| Trabecular separation, μm | 712.8 ± 91.3 | 2469.8 ± 608.2[d] | 298.0 ± 22.7 | 422.9 ± 32.8[e] | 0.004 |
| Longitudinal growth rate, μm/day | 61.7 ± 2.1 | 62.2 ± 3.0 | 59.2 ± 1.2 | 60.9 ± 1.2 | 0.74 |

[a]Static histomorphometry (zone 2) and longitudinal growth rate of proximal tibial metaphyseal cancellous bone after rats were sacrificed on day 21. Group A = age-matched control, group B = cyclosporin A (CsA; 15 mg/kg/day by gavage), group C = prostaglandin $E_2$ ($PGE_2$; 6 mg/kg/day by subcutaneous injection), group D = CsA and $PGE_2$. All values = mean ± SEM.
[b]$p < 0.05$ versus control.
[c]$p < 0.01$ versus control.
[d]$p < 0.001$ versus control.
[e]$p < 0.001$ versus CsA group.

TABLE 4. DYNAMIC HISTOMORPHOMETRY (ZONE 2)[a]

| | *Control (A)* (n = 11) | *CsA (B)* (n = 7) | *$PGE_2$ (C)* (n = 8) | *CsA and $PGE_2$ (D)* (n = 9) | *Interaction term* |
|---|---|---|---|---|---|
| Labeling perimeter,[b] mm | 2.9 ± 0.5 | 2.2 ± 0.5 | 6.3 ± 0.5[c] | 6.1 ± 0.2[c,d] | 0.66 |
| Labeling perimeter,[e] % | 7.6 ± 1.1 | 15.8 ± 1.8[c] | 9.9 ± 0.4 | 10.8 ± 0.8[f,g] | 0.002 |
| Osteoid perimeter,[b] mm | 3.9 ± 0.9 | 3.0 ± 1.0 | 6.8 ± 1.2 | 8.4 ± 1.0[g,h] | 0.23 |
| Osteoid perimeter,[e] % | 9.4 ± 1.1 | 18.9 ± 2.0[c] | 10.5 ± 1.7 | 14.6 ± 1.4[f] | 0.09 |
| Eroded perimeter, mm | 1.4 ± 0.3 | 1.1 ± 0.3 | 4.1 ± 0.1[c] | 6.2 ± 0.6[c,d] | 0.003 |
| Eroded perimeter,[e] % | 3.6 ± 0.4 | 7.1 ± 1.0[c] | 6.7 ± 0.6[h] | 10.6 ± 0.6[c,d] | 0.8 |
| Adjusted mineral apposition rate,[b] μm/day | 1.0 ± 0.2 | 1.2 ± 0.2 | 1.6 ± 0.2[f] | 1.1 ± 0.1 | 0.06 |
| Bone formation rate (BFR/BV),[i] %/year | 86.2 ± 14.0 | 231.7 ± 36.2[c] | 163.3 ± 23.4[f] | 136.5 ± 16.4[g] | 0.0009 |
| Bone formation rate (BFR/BS),[e] μm/day × 100 | 6.0 ± 1.0 | 18.0 ± 2.5[c] | 15.4 ± 2.0[f] | 11.7 ± 1.3[f,j] | 0.0001 |
| Bone formation rate (BFR/TV),[b] %/year | 7.4 ± 1.2 | 8.6 ± 2.7 | 35.3 ± 4.6[c] | 20.4 ± 1.8[f,j] | 0.01 |
| Eroded to labeling perimeter ratio | 0.6 ± 0.1 | 0.5 ± 0.1 | 0.7 ± 0.1 | 1.0 ± 0.1[d,h] | 0.02 |

[a]Dynamic histomorphometry (Zone 2) of proximal tibial metaphyseal cancellous bone after rats were sacrificed on day 21. Group A = age-matched control, group B = cyclosporin A (CsA; 15 mg/kg/day by gavage), group C = prostaglandin $E_2$ ($PGE_2$; 6 mg/kg/day by subcutaneous injection), group D = CsA and $PGE_2$. All values = mean ± SEM.
[b]Tissue-based parameters.
[c]$p < 0.001$ versus control.
[d]$p < 0.001$ versus CsA group.
[e]Bone surface-based parameters.
[f]$p < 0.05$ versus control.
[g]$p < 0.01$ versus CsA group.
[h]$p < 0.01$ versus control.
[i]Bone area-based parameters.
[j]$p < 0.05$ versus CsA group.

mate of bone formation rates than the bone and surface-based values. The bone and surface-based bone formation rates estimated bone turnover rates more than bone formation if one assumed bone formation was equal to resorption. In Table 4, the CsA treatment alone turned over bone more rapidly than $PGE_2$-, $PGE_2$ + CsA-treated, and control bones. However, the CsA value was an underestimation because CsA treatment caused bone resorption to exceed formation.

In agreement with previous reports, the longitudinal growth rate was no different from that in controls in the CsA-treated rats,[6] indicating that CsA, despite its severe deleterious effects on bone, did not affect longitudinal bone growth. Clinically, in accordance with this, children receiving CsA following kidney transplantation appear to reach the normal adult height.[37]

Therapy with $PGE_2$ or CsA alone produced similar elevations in serum BGP compared to control, and the concurrent administration of both CsA and $PGE_2$ resulted in an even higher serum BGP than in all other groups on days 7 and 14. Because serum BGP reflects osteoblastic activity and overall bone turnover, this probably indicates that greater enhancement of osteoblast activity occurred in the combination group. Thus the addition of $PGE_2$ to CsA therapy allows the osteoblast to surpass osteoclastic bone resorption, converting the high-turnover bone loss into high-turnover bone formation. This is supported by the evidence of significantly increased percentage trabecular bone area in the combination group, even with evidence of increased bone resorption (E.Pm), and BGP on day 21 was strongly correlated with percentage trabecular bone area ($r = 0.49$, $p < 0.01$), trabecular width ($r = 0.63$, $p < 0.001$), BFR/BS ($r = 0.55$, $p < 0.01$), and BFR/TV ($r = 0.55$, $p < 0.01$), supporting these data. $PGE_2$ is thought to stimulate osteoblast differentiation and osteoblast activity,[15,20,36] a postulate supported by the finding of increased percentage labeling perimeter and mineral apposition rate compared to control in the present study.

In addition to the changes in bone architecture and morphometry, CsA stimulated 1,25-$(OH)_2$D biosynthesis. This confirms what we found previously.[6,7,38] However, the combination of CsA and $PGE_2$ and $PGE_2$ alone did not alter 1,25-$(OH)_2$D levels. This is in contrast to previous work that reported that $PGE_2$ stimulates the production of 1,25-$(OH)_2$D in vivo in rats[39,40] and in vitro in isolated kidney cells.[41] The in vivo studies, however, used continuous infusions of low-dose $PGE_2$. In humans, elevated levels of $PGE_2$ are believed to be the cause of hypercalcuria and elevated 1α-hydroxylase activity in Bartter's syndrome.[42] The significance of $PGE_2$ in our studies reducing the CsA-induced elevation in 1,25-$(OH)_2$D remains to be clarified. Despite failing to elevate 1,25-$(OH)_2$D, $PGE_2$ elevated BGP levels. This may indicate that CsA and $PGE_2$ stimulate the osteoblast via different mechanisms.

Both CsA and $PGE_2$ have been found to have different effects on bone in vivo and in vitro. CsA inhibits basal and calcemic hormone-induced calvarial bone resorption in vitro,[43,44] in direct contrast to its in vivo effects. CsA is postulated to inhibit the endogenous production of prostaglandins, possibly owing to the inhibition of the release of arachidonic acid precursors from the cell wall.[23] The relevance of this is uncertain, but one could speculate that this inhibition plays a role in the pathogenesis of CsA-induced bone disease, as $PGE_2$ actually alleviated CsA-induced bone disease. This issue is clouded by the supraphysiologic concentrations of $PGE_2$ used in this study. There are data to support lower doses of $PGE_2$ being effective in stimulating bone formation, however,[15,16,18] and a dose-response study using various doses of $PGE_2$ with CsA may be needed to obtain the lowest effective dose of $PGE_2$ for alleviating CsA-induced bone disease. The dose of $PGE_2$ used in the present study was based on previous work demonstrating a maximum anabolic effect with 6 mg/kg/day.[15,18] The stimulation of bone formation or resorption by $PGE_2$ in vivo could be dependent on the local physiologic concentration of other factors, such as cortisol.[9] In vivo, an elevation in BGP following $PGE_2$ administration has been shown in dogs,[20] and this was confirmed in the present study in rats. Conversely, in vitro exogenous $PGE_2$ is reported to inhibit the stimulatory effects of 1,25-$(OH)_2D$ on BGP secretion from human osteoblast-like cells in a dose-dependent fashion.[45] Additionally, interleukin-1$\beta$ is reported to stimulate cellular proliferation and the synthesis of $PGE_2$ but to antagonize BGP production in cultured human osteoblast-like cells.[46] These conflicting in vivo and in vitro results could reflect the biphasic effects of the prostaglandins.

This study has clinical relevance because reports of bone histology in renal transplant patients[47,48] and of bone density in patients following cardiac and renal transplantation[49,50] have shown bone loss in patients receiving CsA and other immunosupporessive agents. The present study indicates that $PGE_2$ therapy may have a beneficial effect in patients receiving CsA. Preliminary results of clinical trials investigating the effect of $PGE_1$ on CsA-induced renal damage in posttransplantation patients have been published.[24]

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